SIMULATION AND OPTIMIZATION OF SIMULTANEOUS FERMENTATION AND SEPARATION OF BUTANOL USING PERVAPORATION TECHNIQUE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Bachelor of Technology

in

Chemical Engineering

By

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RASHMI RANJAN SAHOO(10300005)

Department of Chemical Engineering
National Institute of Technology, Rourkela
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CERTIFICATE

This is to certify that the thesis entitiled, "**Simulation and Optimization of Simultaneous Fermentation and Separation of Butanol using Pervaporation Technique"** submitted by Sri Aby. J. Kottukappally and Sri Rashmi Ranjan Sahoo in partial fulfillment of the requirements for the award of Bachelor of Technology Degree in Chemical Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by them under my supervision and guidance.

To the best of my knowledge the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree/Diploma.

Date

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Acknowledgement

We express our sincere gratitude to **Dr. G.R.Satpathy**, Professor of the Department of Chemical Engineering, National Institute of Technology, Rourkela, for giving us this great opportunity to work under his guidance throughout the course of this work. We are also thankful to him for his valuable suggestions and constructive criticism which have helped us in the development of this work. We are also thankful to his optimistic nature which has helped this project to come a long way through.

We are also thankful to **Sri Harmohan Jena,** Lecturer, and Department of Chemical Engineering for his assistance in the project work for his constructive criticism.

We are also thankful to the **Prof (Dr.) Pradip Rath**, Head of the Department and our Department for providing us the necessary opportunities for the completion of the project.

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Abstract

Product inhibition continues to be one of the major problems impeding the continuous production of butanol by fermentation process. Although many techniques are available which could sort out the fore mentioned problem , pervaporation continues to be the front runner in terms of effectiveness and efficiency. Analysis of biochemistry of ABE fermentation leads us to the development of new solvent-producing strains and changes in the metabolic pathway which could lead to significant amount of solvents production without the product inhibition problem. In this project we select a well-defined model for the fermentation process and try to reproduce the results of the pervaporation process. The set of differential equations were analyzed for their consistency and were solved using ode45 solver in Matlab. For the process involving pervaporation, a pervaporation factor (rp) was taken into account. This factor was derived from the data of the fermentation process so that accurate representation is possible. There were several constraints in choosing this factor. The inherent factors of the system such as scaling up on the retentate side, which decreased the effective area available for pervaporation, were not considered. It was also found that the rp factor had a pronounced effect, when even changed slightly, on the substrate consumption rate and the biomass production. The butanol rate was affected only when the factor was changed drastically. After several trial runs we are led to the conclusion that we should have different values for rp in the two systems so as to give a realistic representation of the model. The biomass production in both the systems reached the same maximum concentration, a fact supporting the model.

Chapter 1

INTRODUCTION

Background

1.1 Background

Fermentation is an attractive method for production of chemicals and fuels from renewable resource. Unfortunately, fermentative products are present at low concentrations and conventional separation methods are inefficient and energy consuming. Therefore, development of economical processes for product recovery from fermentation broth is necessary. The production of butanol by acetone–butanol–ethanol (ABE) fermentation used to be one of the largest bioprocesses until the 1950s, but later it was replaced by the less expensive petroleum-based chemical synthesis. Although the microbial production is still considered a potential source of fuels, the process suffers from two major impeding problems which are:

- Low productivity of strain involved
- Severe product inhibition by butanol and acetone

The maximum concentration of total solvents does not typically exceed 20 g/l in a batch reactor with typical a weight ratio of 3:6:1 of acetone, butanol and ethanol respectively. This means that one must remove 980 gms of water from one liter of fermentation broth in order to obtain 20 g/L of pure solvent, out of which butanol is about 8-13 g/L which results in high cost solvent recovery in industrial plants. The low concentration of the fermentative products means not only a cost intensive product separation but also a large volume for downstream processing and waste water treatment. Since butanol is less volatile than water, the separation of butanol from dilute aqueous solutions by distillation is unfavorable; it is estimated that at a butanol concentration of <5%, the energy consumption required for butanol purification will exceed the energy content of the butanol recovered.

As an alternative to distillation, several techniques for solvent removal has been investigated in order to reduce the butanol inhibition and enhance solvent productivity and sugar utilization.

There are two methods to economize butanol production commercially:

- Genetic manipulation of the butanol producing strain to produce and tolerate high concentration of butanol
- Process development for simultaneous production and recovery

Genetic manipulation of the bacterium involved is proven to be difficult and involves considerable economics making the process unable to compete with petroleum based products. As a result process development has received much more attention and marked improvements in productivity has been reported following the application of novel

technologies such as continuous culture and immobilized cells. However, these productivity gains have often been made at the expense of product concentration and sugar utilization. The use of continuous flow reactors for the fore-mentioned fermentation process has led to problems like

- Product recovery from dilute solutions
- Disposal of large amounts of reactor effluent

One solution to above problems is to recycle the reactor effluent and to achieve complete sugar utilization and higher product concentration, but problems of product inhibition would normally preclude this. The concept of integrated fermentation /product recovery process is one which allows continuous removal of inhibitory product from a reactor and there by increasing reactor productivity. This concept has been applied to ABE fermentation process using a variety of product recovery processes like

- Adsorption
- Gas stripping
- Pervaporation
- Liquid-liquid extraction
- Perstraction
- Reverse osmosis
- Membrane distillation
- Chemical recovery
- Salt induced phase separation

Chapter 2

PERVAPORATION

Introduction

Pervaporation

Membranes

Applications

Why Pervaporation in ABE?

2.1 Introduction

Pervaporation, in its simplest form, is an energy efficient combination of membrane permeation and evaporation. It's considered an attractive alternative to other separation methods for a variety of processes. For example, with the low temperatures and pressures involved in pervaporation, it often has cost and performance advantages for the separation of constant-boiling azeotropes. Pervaporation is also used for the dehydration of organic solvents and the removal of organics from aqueous streams. Additionally, pervaporation has emerged as a good choice for separation heat sensitive products. Pervaporation can used for breaking azeotropes, dehydration of solvents and other volatile organics, organic/organic separations such as ethanol or methanol removal, and wastewater purification.

2.2 Pervaporation

Pervaporation involves the separation of two or more components across a membrane by differing rates of diffusion through a thin polymer and an evaporative phase change comparable to a simple flash step. A concentrate and vapor pressure gradient is used to allow one component to preferentially permeate across the membrane. A vacuum applied to the permeate side is coupled with the immediate condensation of the permeated vapors. Pervaporation is typically suited to separating a minor component of a liquid mixture, thus high selectivity through the membrane is essential. Figure 2.1 shows an overview of the pervaporation process

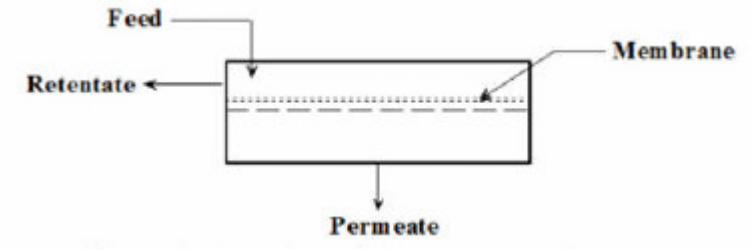

Fig 2.1 – Overview of the Pervaporation Process

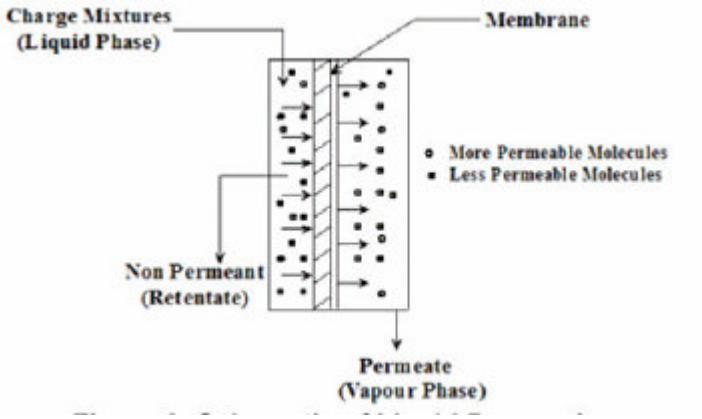

Fig 2.2 – Schematic of Liquid Permeation

Liquid transport in pervaporation is described by various solution-diffusion models[1]. The steps included are the sorption of the permeate at the interface of the solution feed and the membrane, diffusion across the membrane due to concentration gradients (rate determining steps), and finally desorption into a vapor phase at the permeate side of the membrane. The first two steps are primarily responsible for the permselectivity. As material passes through the membrane a "swelling" effect makes the membrane more permeable, but less selective, until a point of unacceptable selectivity is reached and the membrane must be regenerated. The other driving force for separation is the difference in partial pressures across the membrane. By reducing the pressure on the permeate side of the membrane, a driving force is created. Another method of inducing a partial pressure gradient is to sweep an inert gas over the permeate side of the membrane. These methods are described as vacuum and sweep gas pervaporation respectively.

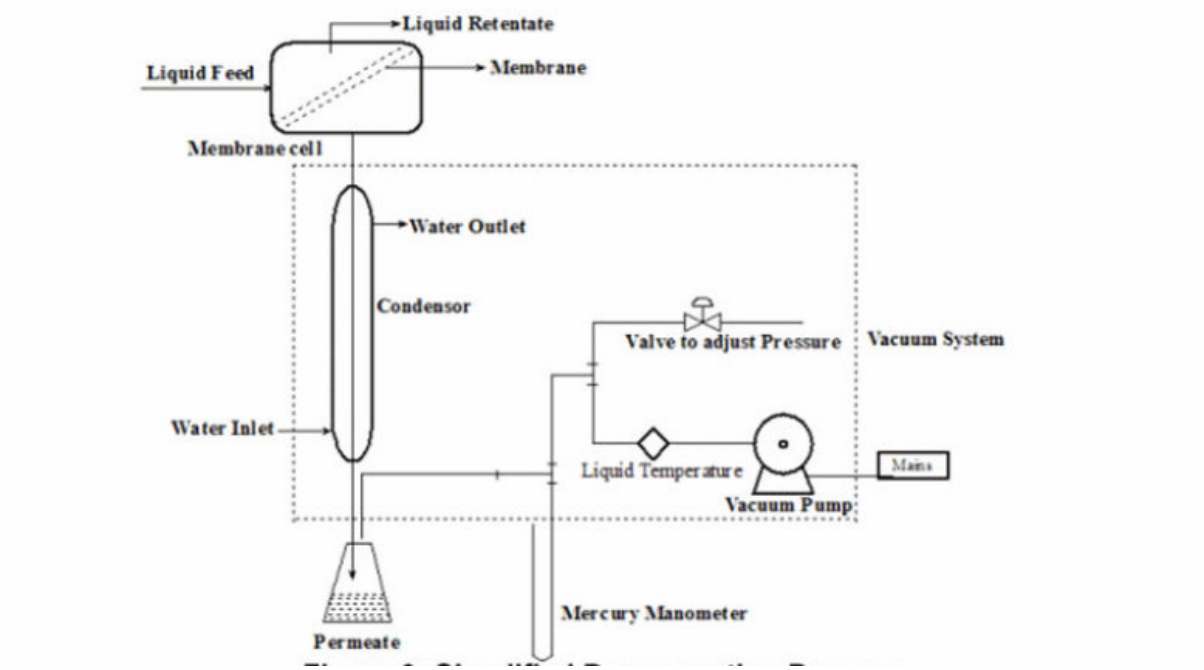

Fig : 2.3 – Basics of the Pervaporation Setup

The pervaporation of dilute organic-water mixtures has been relatively well described by a resistance-in-series model. The mass transfer process of a single component across the membrane occurs in 4 consecutive steps:

1- Mass transfer from the bulk of feed to the membrane interface.

2- Selective absorption into the membrane at the feed side.

3- Selective diffusion through the membrane.

4- Desorption into the vapor phase at the permeate side.

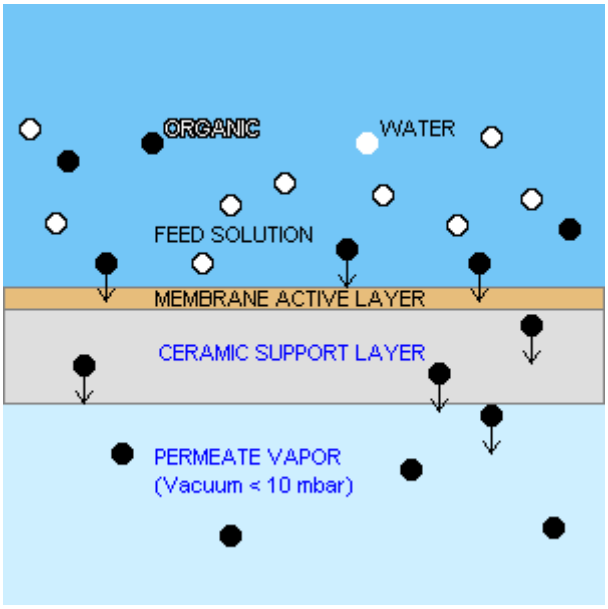

Fig 2.4 – Resistance to pervaporation in series

Although membrane materials govern the pervaporation process and determine the separation factor, operational conditions do effect the permeation flux and selectivity:

1- Temperature: This parameter effects the process in all steps. It can be modeled by an Arrhenius expression: $J = J_o e^{(-Ea/RT)}$. Generally, flux increases with temperature due to kinematics viscosity. Too high a temperature may result in increasing hydraulic resistance.

2- Pressure: As mentioned above, vapor difference is the driving force for pervaporation. The larger the transmembrane pressure difference is, the larger is the permeate flux. However, a large pressure difference can result in no effect or in fouling of the membrane.

3- Cross flow velocity: Within small Reynolds number range, flux can be enhanced by reducing hydraulic diameter or by increasing flow velocity. A critical velocity is necessary to achieve the maximum separation potential. However, in turbulent flow regimes, very small flow effect is observed.

4- Feed concentration: Since overall permeability of a component depends on its solubility and diffusion rate through the membrane, the permeate flux is significantly influenced by feed concentration.

The characteristics of the pervaporation process include:

1. Low energy consumption
2. No entrainer required, no contamination
3. Permeate must be volatile at operating conditions
4. Functions independent of vapor-liquid equilibrium

2.3 Membranes

The membranes used in pervaporation processes are classified according to the nature of the separation being performed. *Hydrophilic membranes* are used to remove water from organic solutions. These types of membranes are typical made of polymers with glass transition temperatures above room temperatures. Polyvinyl alcohol is an example of a hydrophilic membrane material. *Organophilic membranes* are used to recover organics from solutions. These membranes are typically made up of elastomer materials (polymers with glass transition temperatures below room temperature). The flexible nature of these polymers makes them ideal for allowing organic to pass through. Examples include nitrile, butadiene rubber, and styrene butadiene rubber

Different types of membranes used are:

- Polydimethylsiloxane (PDMS) membranes
- polypropylene(PP) membranes
- polytetrafluoroethylene(PTFE) membrane
- poly[1-(trimethylsilyl)-1-propyne] (PTMSP) membrane
- poly(ether block amide) (PEBA)
- Styrene butadiene rubber (SBR)
- Silicalite-silicone membrane
- Silicone membrane

PDMS is the most widely used organophilic membrane material. PTMSP, which is a glassy polymer with a large free volume, were also found to be selective to organic compound permeation. Hydrophobic PP and PTFE do not exhibit a high selectivity as the separation is based on the flow of the ABE and water vapors through the pores of the membrane.

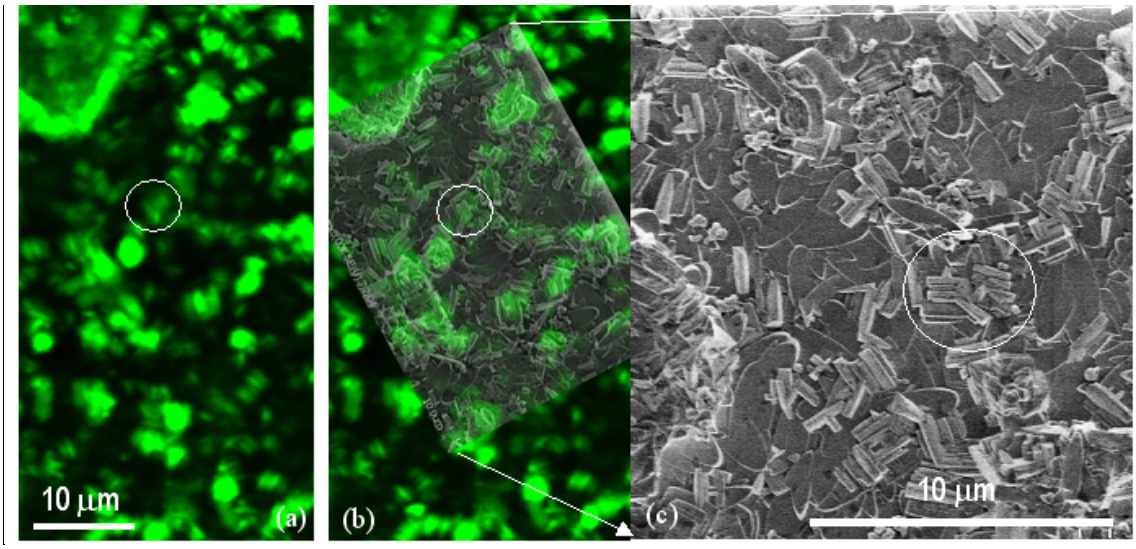

Fig 2.5 : Picturization of membranes on micrometer scale

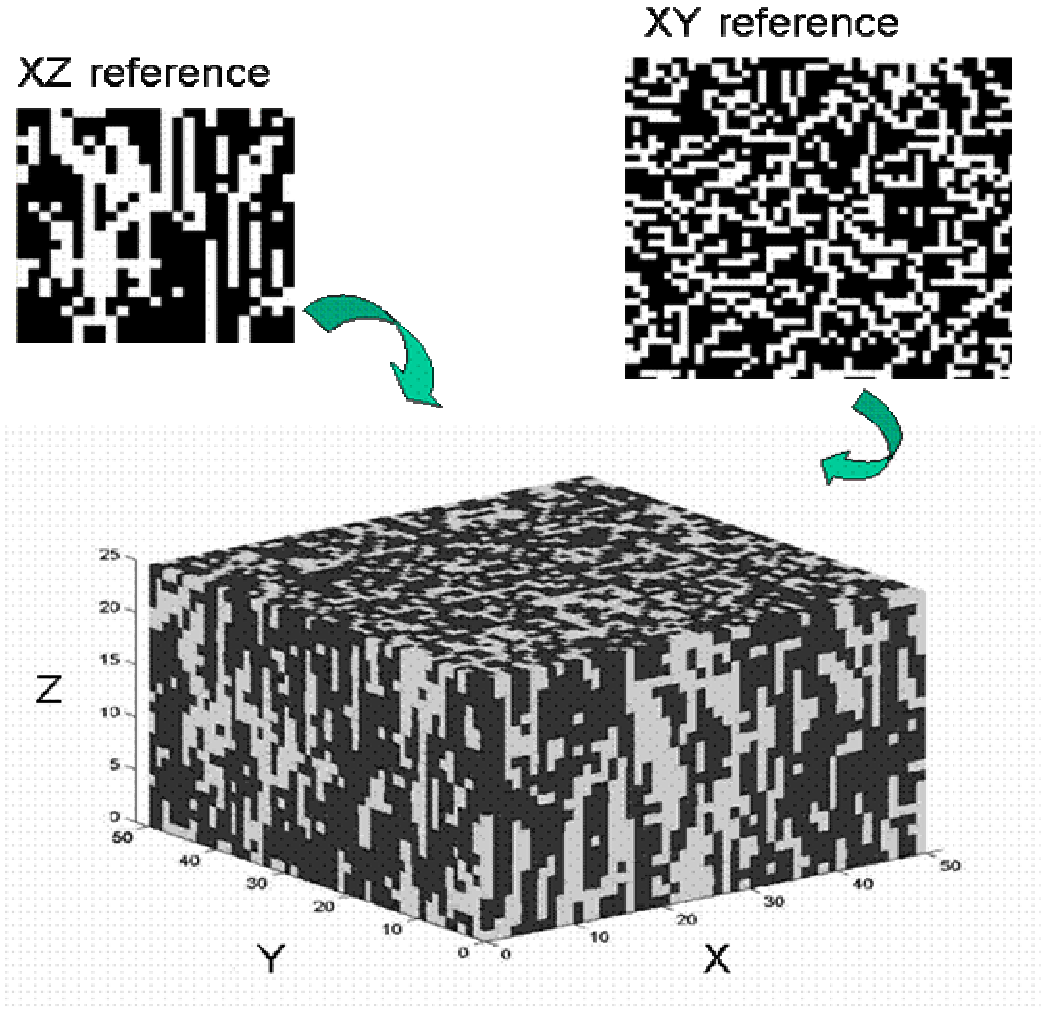

Fig 2.6 :- Axial view of surface of pervaporation membrane

2.3.1 Factors Affecting Membrane Performance

According to the solution-diffusion model, higher fluxes can be obtained with an increased thermal motion of the polymer chains and the diffusing species. Properties of the polymers that affect diffusion include the "backbone" material, degree of cross-linking, and porosity. A molecular-level interaction between membranes and diffusing species is expressed via a permeability constant used in the Arrhenius relationship:

$$P = P_o e^{-E_p/RT} \quad (1)$$

Where,
E_p = Activation energy
P_o = Permeability constant
R = Gas constant
T = Temperature

2.3.2 Membrane Characteristics

1. Molecular Flux

Molecular flux is the amount of a component permeated per unit area per unit time for a given membrane.

$$J_i = Q_i / (At) \quad (2)$$

Where,
J_i = Flux of component "i" (moles/h cm^2)
Q_i = Moles of component "i" permeated in time "t"
A = Effective membrane surface area (cm^2)

2. Permselectivity

The performance of a given membrane can be expressed in terms of a parameter called permselectivity:

$$\alpha = (X_i^p/X_j^p)/(X_i^f/X_j^f) \quad (3)$$

$$\alpha = (V_i^p\rho_i^p/V_j^p\rho_j^p) / (V_i^f\rho_i^f/V_j^f\rho_j^f) \quad (4)$$

Assuming the density of the components in the feed is the same, then:

$$\alpha = (V_i^p/V_j^p)/ (V_i^f/V_j^f) \quad (5)$$

Where,
X = Weight fraction
V = Volume fraction

p = Density
Superscripts "p" and "f" denote "permeate" and "feed" respectively while "i" and "j" represent individual components.

3. Permeability Coefficient

The molecular flux for pervaporation across a membrane can be related to the permeability coefficient by:

$$J_i = -P_i \Delta^{p/l} \quad (6)$$

or

$$J_i = k \Delta^{p/l} \quad (7)$$

Here, $\Delta P = P_1 - P_2$ and $P_1 = P_i^{\circ} X_{r,i,\gamma i}$ & $P_2 = P\, Y_{p,i}$, therefore

$$\Delta P = (P_i^{\circ} X_i - P\, Y_{p,i}) \quad (8)$$

Equation 6 becomes,

$$J_i = - p_i (P_i^{\circ} X_{r,\,i} - P\, Y_{p,\,i})/L \quad (9)$$

$$P_i = -J_i L/(P_i^{\circ} X_{r,\,i,\,\gamma i} - P\, Y_{p,i}) \quad (10)$$

Pi = Permeability coefficient of component 'i'.
γ_i = Activity coefficient of component 'i' in the mixture.
ΔP = Change in partial pressure of pure component 'i' across the membrane.
P_i° = Saturation pressure of pure component 'i' at feed temperature.
$X_{r,}i$ = Mole fraction of component 'i' in liquid feed.
$Y_{p,I}$ = Mole fraction of component 'i' in permeate.
Ji = Flux of component 'i'.
L = Membrane thickness.
$k = P_i/L$ = Permeability constant.

2.4 Applications

1 .The treatment of wastewater contaminated with organics

2 .Pollution control applications

3 .Recovery of valuable organic compounds from process side streams

4 .Separation of 99.5% pure ethanol-water solutions

5 .Harvesting of organic substances from fermented broth

2.5 Why Pervaporation in ABE ?

Pervaporation is considered to be the best among above techniques to remove butanol from fermentation broth because it does not cause any harmful effects on the microorganisms and is potentially less expensive than distillation. Additional advantage of pervaporation is that it doesn't affect the culture. Pervaporation always results in a concentrated product stream. When coupled to fermentation, pervaporation has resulted in the improvement of productivity, yield and sugar utilization due to reduction of end product inhibition.

Chapter 3

ABE FERMENTATION

What is ABE fermentation?

Importance of ABE fermentation

Why biochemistry of ABE fermentation is important?

Biochemistry

Types of microorganisms and their strains

Applications

3.1 What is ABE fermentation ?

ABE stands for acetone – butanol – ethanol , the major solvents which are produced during the fermentation employing clostridium bacteria . although this process had been around for pretty long time , it was not until 1990's it was developed on a commercial scale. The importance of this process lies in the fact that it produces three solvents viz. acetone, butanol, ethanol and two gases viz. hydrogen and carbon dioxide along with traces of byproducts. The primary concern of any industry is the optimum substrate utilization which still remains a problem in the above fermentation process. Experiments are still underway in understanding the biochemistry to the atomic level so that we could regulate the formation of products thus making it more flexible..

3.2 Importance of ABE fermentation

The reason for high level of interest in basic research and development of ABE fermentation is straight forward.

- First , although there is no immediate threat that the world's petroleum reserves would be used up , a severe dwindling of these non renewable resources , which serve as the major source of fuels and chemicals , will most likely occur in the mid – twenty century.
- Second, it is now technologically possible to produce essentially all commodity chemicals from renewable biomass feed stocks such as starch and cellulose.
- Third, from about 1915 until the mid-1940s, the ABE fermentation made a significant impact on commercial solvent production. Typically, more than 1000 kg of butanol, 500kg of acetone and 175 kg of ethanol plus other utilizable by-products were produced in 90,000-L fermentors in N. America and elsewhere.
- Fourth, biomass- derived solvents produced by ABE fermentation can enter into the current petrochemical synthetic pathways through number of reactions. The most important of these is the dehydration of alkanols to alkene to form ethylene, propylene, butylenes, and butadiene.

3.3 Why biochemistry of ABE fermentation is important?

To obtain a high yield of a specific product in fermentation for solvents, it is often necessary simply to adjust the growth medium, carbon source, and other conditions that will

ensure maximum amounts of the desired solvent and minimize production of other products. However research on cellular regulatory mechanisms that govern the fermentation not only will allow more effective control of culture conditions but will open door to development of highly productive strains. Because many *Clostridial* fermentations typically depend on branched fermentation pathways, analysis of control elements becomes particularly important. We are also concerned with the culture conditions that promote the shift away from acid production and favor optimal butanol and acetone production. The study of biochemistry also reveals the prominence of product inhibition, if present which would enable us to preset conditions, which would result in optimum utilization of substrate.

3.4 Biochemistry

There are three major groups of compounds that come inot picture during the ABE fermentation process which are

- Adenosine phosphates (AMP ,ADP , ATP), which link energy yielding and energy requiring reactions
- Nicotinamide adenine dinucleotide (NAD , NADH)
- Nicotinamide adenine dinucleotide phosphate (NADP , NADPH)

The Embden-Meyerhof-Parnas (EM) pathway, the pentose-phosphate (PP) pathway and the Entner-Doudroff (ED) pathway are the three routes for the utilization of hexoses such as glucose. The EM and PP pathways are widespread in fungi and bacterial cells. The ED pathway is common in bacteria and in some fungi such as *Tilletia caries* and *Caldariomyces fumago*

3.4.1 *Clostridium's* general fermentation strategy

Thauer et al outlined the general energy metabolism of the chemotrophic anaerobic bacteria, which include the *Clostridia.* The *Clostridium* depends almost exclusively on the fructose – biphosphate pathway (Embden-Meyerhof-Parnas (EM) pathway) for the conversion of one hexose to two pyruvates with the net production of two ATPs and two NADHs. In the fermentation of pentose, the intermediates from 3 mol of pentose – 5-phosphate, are one glyceraldehyde 3-phosphate and two fructose 6-phosphates. A combination of the enzymes transaldolase and transketolase is used. The sugar-phosphates enter the fructose bi-phosphate pathway and have capability of producing five ATPs and five NADHs per 3 mol of pentose fermented.Yet NADPH-ferredoxin oxidoreductase which is ubiquitous in all clostridia investigated, probably provides the NADPH for biosynthesis

during both vegetative growth and sporulation. Most pyruvate produced from sugars during the *Clostridial* fermentation is cleaved by pyruvate-ferredoxin oxidoreuctase in a coenzyme A (CoA) dependent reaction yielding CO_2 , acetyl CoA , and reduced ferredoxin (Fd_{red}). Acetyl CoA is central to all *Clostridial* fermentations. The thioester bond of acetyl CoA is a very high energy bond ; thus it is an important source of ATP in most *Clostridial* fermentations , because for every mole of butyrate or acetate produced , a mole of ATP is formed. Acetyl CoA is also the most important precursor of all alcohols and organic acids synthesized by these organisms; it thus serves as a major intermediate in these fermentations. The NADH, which is formed during oxidation of glucose is thus reoxidised and recycled so that fermentation can continue.

3.4.2 Butanol fermentation

(Refer Fig 3.1)

In a typical experimental batch culture fermenting glucose, there is an early accumulation of acids (acidogenic phase) followed by butanol formation (solventogenic phase) and a reutilization of butyrate and acetate. C. acteobutytcium growing exponentially on sugars or starch at a pH 5.6 or greater produces butyrate, acetate, CO_2, and H_2 as the major fermentation products. During this acidogenic fermentation phase, the bacteria are running a basic butyrate-acetate fermentation. To maintain electron balance, for every mole of acetate formed , an extra mole of H_2 is formed. Therefore, about 2.4 mol of H_2 is produced from a fermentation of 1 mol of hexose that produces 0.4 mol of acetate and 0.8 mol of butyrate. The extra 0.4 mol of H_2 comes from transfer of electrons from NADH through NADH: Fd oxidoreductase and hydrogenase to H_2. With accumulation of acids in a batch culture, the pH drops to pH 4.0 - 4.5 (depending on the strain), the growth becomes linear, and the classical switch in the fermentation occurs. The triggering of solvent formation requires the induction of new enzyme pathways in the cells catalyzing formation of butanol, acetone and ethanol. During this phase , there is a net uptake of butyrate and acetate from the fermentation beers back to the cells and their recycling and conversion to butanol or ethanol. Finally , acetone or isopropanol is produced as a result of decarboxylation of acetoacetate .

Depending on the Clostridial strain, one or two CoA- dependent aldehyde dehyrdogenases and two or three alcohol dehydrogenases are needed for butanol, ethanol, and isoprpanol formation. Three additional changes occur as a result of the shift from acidogenesis to solventogenesis :

- There is a lower ATP yield per mol of glucose fermented because net accumulation of acetate and butyrate allows additional production from acetyl phosphate and butryl phosphate during exponential growth.
- Operation of the alcohol pathways requires 2 mol additional NADH per mol of alcohol produced.
- The amount of H_2 made is reduced to about 1.4 mol of H_2 per mol of glucose fermented ,since electrons must be shunted from FdH_2 to NADH .These changes in the electron flux may be involved in regulatory signals for promoting the induction of the shift .

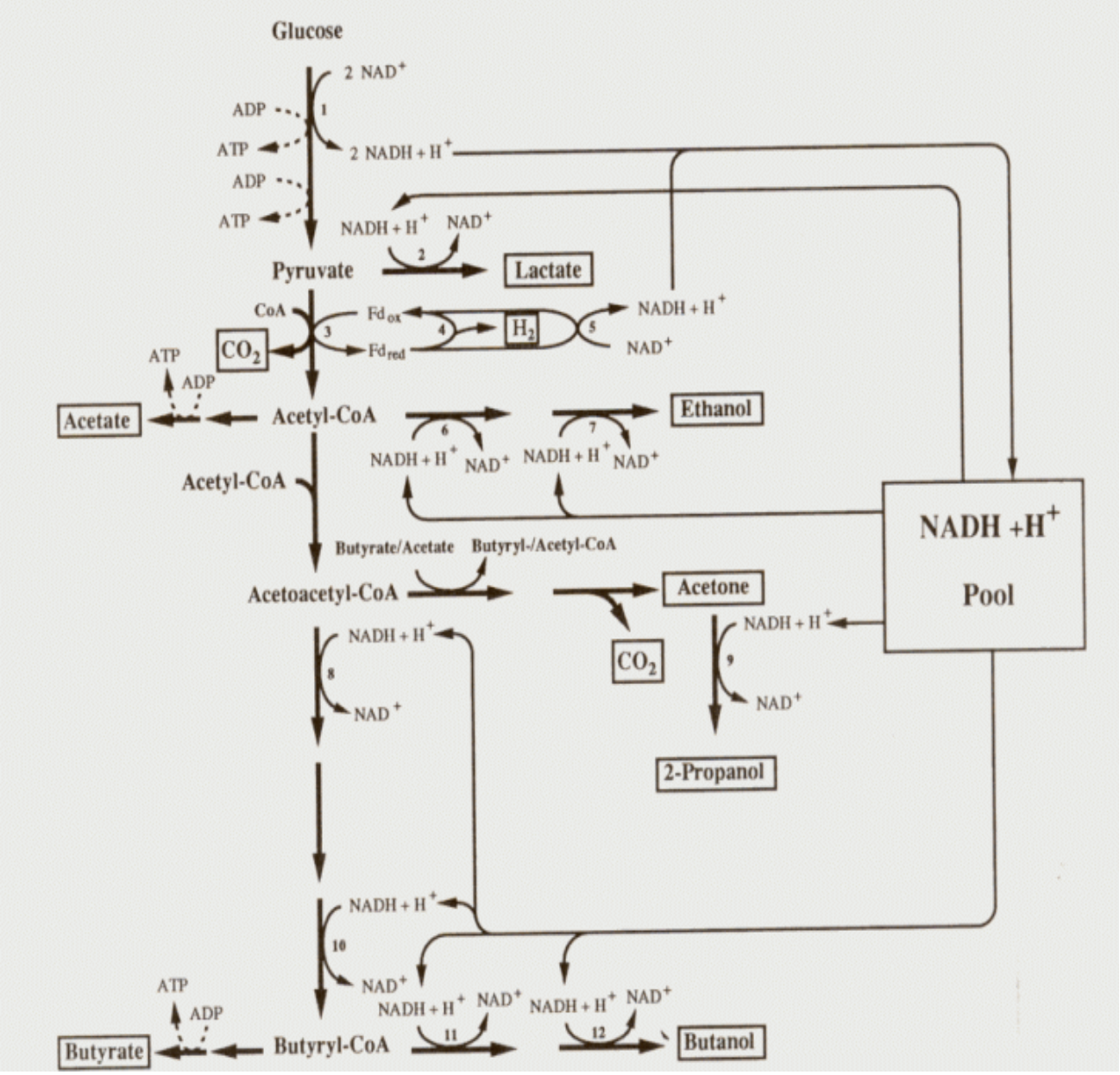

Fig 3.1 :- Butanol fermentation

Acetone and eventually isopropanol production requires two enzymes: acetoacetyl-CoA transferase (ACT) and acetoacetate decarboxylase. The production of acetone by many of the solvent forming *Clostridia* is biologically interesting because the pathway neither produces extra ATP nor act as an electron sink. After induction of these enzymes , acetate and butyrate are taken up by the bacteria , the ACT enzyme transfers CoA from acetoacetyl-CoA to these acids to form their acyl-CoA intermediates and then they are funneled through alcohol pathways to butanol and ethanol. The decarboxylase pulls the reaction forming CO_2 and acetone or is reduced to iso-propanol and excreted. During the solvent production phase, reduction in concentration of acids in the medium occurs early while acetone continues to accumulate long after, when there is no net increase in acids. It appears that in these *Clostridia*, acid production and recycling occur throughout solvent formation.

3.4.3 Embden-Meyerhof-Parnas (EM) pathway

(Refer Fig 3.2)

This pathway involves ten enzyme-catalysed steps which start with glucose and end with pyruvate. Examination of the EM pathway shows that each step is quite simple and involves isomerisation, ring splitting or transfer of a small group such as hydrogen or phosphate .Two moles of pyruvate are produced per mol of glucose passing through the pathway. ATP hydrolysis is coupled with two reactions which would not occur otherwise, and two reactions involve sufficiently negative free energies to drive ADP phophorylation. Because the latter two reactions occur twice for each mol of glucose processed, the overall effect is phosphorylation of ATP.Dehydrogenation of glyceraldehyde 3-phosphate is coupled with the reduction of NAD^+ , and this reaction occurs twice per mol of glucose. Thus, the overall stoichiometry of the EMP pathway is

$$C_6H_{12}O_6 + 2\,P_i + 2\,ADP + 2NAD^+ \rightarrow 2C_3H_4O_3 + 2\,ATP + 2\,(NADH + H^{+)}$$

Stored chemical energy and reducing power result from the overall pathway .Energy storage accomplished by this or other substrate rearrangement pathway is called substrate level phosphorylation.

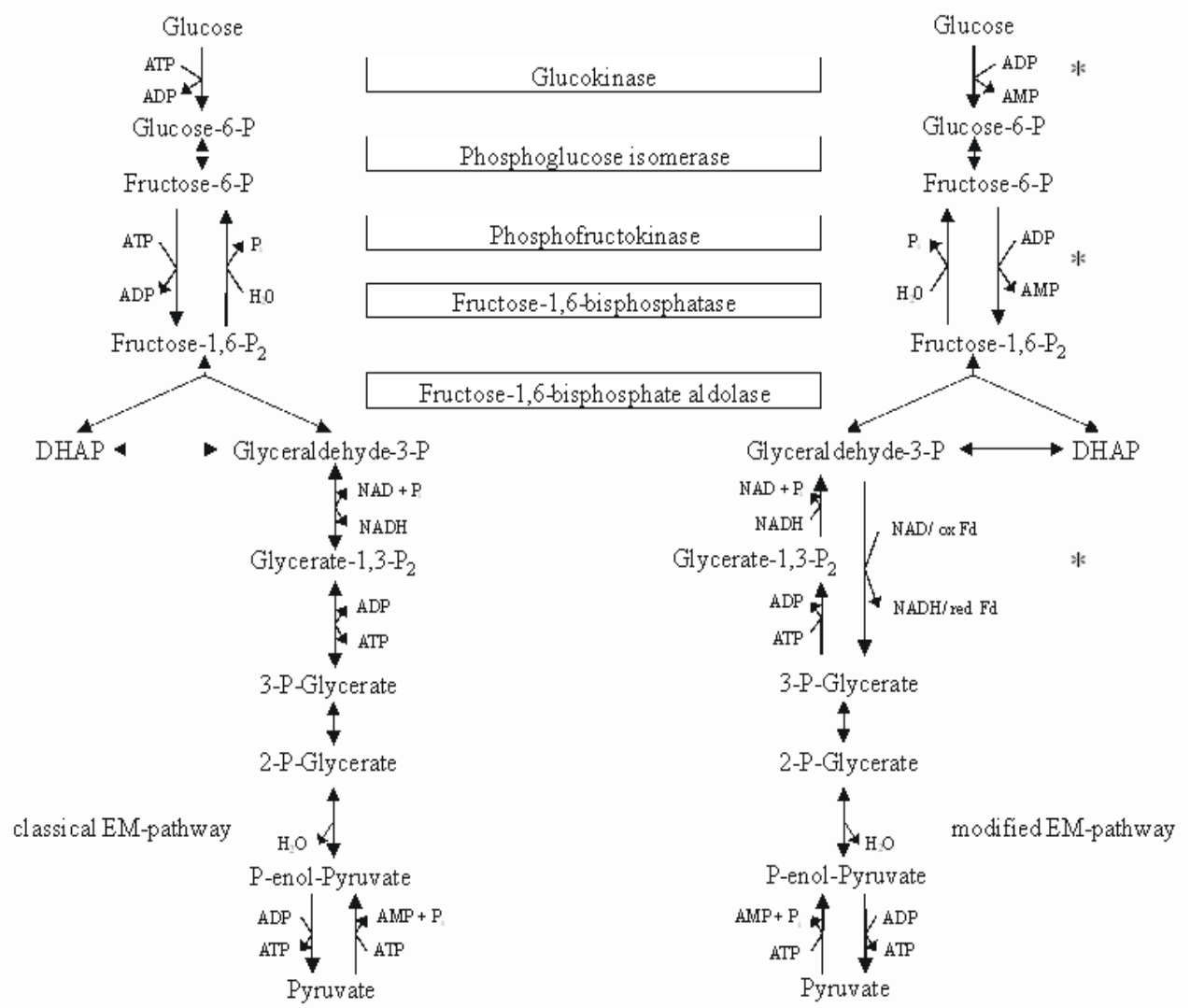

Fig 3.2:- Embden-Meyerhof-Parnas (EM) pathway

3.4.4 Physiology of control of solvent formation

The culture conditions that promote the shift away from the acid production and favor optimal butanol and acetone production are the main areas of interest. The conditions that favor butanol and ethanol production without acetone (the alcoholic fermentation) by continous cultures of *C. acetobutylicum* is of high concern as it is a high solvent producing. The internal status of the bacterium for acidogenic , solventogenic, alcoholic fermentations are measured .During the acidogenic fermentation in a batch or continous cultures , the following conditions are required : adequate glucose , a pH greater than 6 ; and a low level of organic acids. The intracellular pH (about 6.8) is maintained close to neutral either by excretion of H_2 through the action of hydrogenase or by hydrolysis of ATP and pumping H^+ out of the cell. At a pH 6.8, the cell maintains a low level of undissociated butyric acid. ATP is produced and utilized at a high rate during this

fermentation stage. The low NADH/NAD ratio is probably as a result of the high NADH: Fd oxidoreductase activity, where by excess electrons are dumped onto FdH_2 and exit through hydrogenase as molecular hydrogen. H_2 is produced in excess in the acidogenic fermentation.

The shift to solventogenic fermentation requires an adequate carbon source carbon source (glucose), an acid pH (4.8 – 4.4) and high concentrations of butyrate and acetate in batch cultures. Continous chemostat cultures limited for PO_4 or nitrogen source can be forced to carry out a solventogenic fermentation either by adding a high concentration of organic acids or by setting the pH to 4.8 – 4.3 ,depending on the strain of *Clostridium*. The intracellular pH is around 5.9 and results in a greater than 10-fold higher concentration of undissociated butyric and acetic acids in the cell. Also a very high ΔpH of 1.1 – 1.5 is maintained by these cells. Curiously, this high ΔpH exists even when solvent producing cells are treated with N, N'- dicyclohexylcarbodiimide (an ATPase inhibitor) or after passing CO gassing (a hydrogenase inhibitor). A high concentration of ATP is maintained and turnover of ATP is slowed in the cells, because growth is slower during solvent production. The NADH/NAD ratio is maintained similar to acidogenic cells, even though production of butanol results in new sites for oxidation of NADH. This is due to net transfer of electrons from FdH_2. This physiologic state is essential for the induction of enzymes required for solvent fermentation.

The alcoholic fermentation, in which high amounts of butanol and ethanol are produced without acetone, in continous cultures requires high glycerol and limited glucose, at a neutral pH.when inhibitors which prevent the formation of acetone are added to the cultures with adequate glucose, causes an overload of NADH in the cell, resulting in a high NADH/NAD ratio and various levels of ATP. The high level of NADH leading to butanol and ethanol formation is accompanied by increased activities of NADH-dependent alcohol and aldehyde dehydrogenases which differ from the NADPH-dependent hydrogenases that are expressed at high levels during solventogenesis in the bacterium

3.5 Types of microorganisms and their strains

Table 3.1 *:- Clostridial* species for Solventogenic fermentations

SL NO	Former names	Proposed names
1	C.thermocellum LQRI, ATCC 35609, DSM 2360	No change (cluster III)

2	C. thermohydrosulfuricum E100-69^{T},ATCC 35045, DSM 567	Thermoanaerobacter thrmohydrosulfuricus
3	C. thermohydrosulfuricum, Thermoanaerobacter ethanolicus JW200^{T,} 39E,ATCC 33223	(cluster V) Thermoanaerobacter ethanolicus (cluster V)
4	C. thermosachharolyticum NCA 3814, ATCC 7956	Misclassified, belongs in genus *Thermoanaerobacter* (cluster V)
5	C. sachharolyticum NRCC 2533, ATCC 35040	No change
6	C. acetobutylicum ATCC 824, 4259 Weizmann strain; DSM 792 and 8 strains	No change, “taxon I”
7	C. acetobutylicum NRRL B643, NCP 262 and four NCP strains	C “taxon II”
8	C. sachharoperbutylacetonium NI-4,NI-504, ATCC 27021	No change, “taxon III”
9	C. beijerinckii, C. acetobutylicum ATCC 25732^{T}, NRRL B 592, NCIMB 9362^{T},8052^{T} and 19 strains	C. beijerinckii, “taxon IV”
10	C. puniceum NCIMB 11596	No change; closely related to taxa II and III
11	C. aurantibutyricum ATCC 17777	No change
12	C. tetanomorphum MG-1, ATCC 49273, DSM 4474	No change

Table 3.2 :-Ethanol and Butanol fermentation by *Clostridium* species

SL NO	Organism	Complex polymer	Substrates utilized
1	C. thermocellum	Cellulose, xylan	Cellubiose,few hexoses and pentoses
2	C. thermohydrosulfuricum	Starch, pectin, salicin, xylan	Many disaccharides, hexoses, pentoses, etc.
3	C. thermosachharolyticum	Starch, xylan	Disaccharides, hexoses and pentoses
4	C. sachharolyticum		Disaccharides, hexoses and pentoses
5	C. acetobutylicum taxon I	Starch, xylan	Cellobiose, lactose, some hexoses and pentoses
6	C. acetobutylicum taxon II		
7	C. sachharoperbutylacetonicum taxon III	Starch	Sucrose, cellubiose, some hexoses and pentoses
8	C. beijerinckii taxon IV	Starch	Sucrose, cellubiose, some hexoses and pentoses
9	C. aurantibutyricum	Starch	Sucrose, hexoses and pentoses
10	C. puniceum	Starch, pectin	Hexoses, pentoses
11	C. tetanomorphum		Cellubiose, some hexoses and pentoses

3.6 Applications

The major applications of the above study include :

- elucidating the physiological signals and details of molecular sensor and responder systems
- product tolerance problem
- biology of substrate utilization
- metabolic pathway inactivation and amplification

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Chapter 4

Process

Overview

Results

4.1 Overview

Batch culture experiments were conducted using modified solvent producing strains which features decreased amounts of butyric acid at the end of fermentation and good solvent production. The culture has undergone a strain selection procedure using butyric acid enriched medium. The medium used was a defined one which contained specific amounts of known nutrients. Although media preparation was a bit expensive from the economic point of view; it is allowed us to have a better control of the fermentation process and produced a reproducible condition. The fermentor used for the above process was slightly agitated to facilitate effective transfer. The temperature was kept near to room temperature with no pH regulation for the above process. The working volume was inoculated with growth phase culture. Fermentation process was carried anaerobically with oxygen free N_2 flow. The bio-mass was measured by optical density of suitable wavelength previously calibrated against dry weight concentration (in g/l). The product of fermentation were determined by gas-liquid chromatography (GLC) using a flame ionizing detector (FID). An inert gas such as nitrogen was used as a carrier so that it doesn't interface with the products being carried. Pervaporation was carried out with oxygen free nitrogen at a specific flow rate and the pervaporation was collected via condensation at low temperature

4.2 Results

According to separation theory, mass transport through a pervaporation membrane is proposed to follow a sorption–diffusion mechanism with sorption of liquid solute into the membrane at the feed side, transport through the membrane, and desorption into the vapor phase at the permeate side of the membrane. Based on this sorption–diffusion mechanism, membrane performance should be enhanced by improving either selective sorption or selective diffusion. Therefore, microporous absorbent with high sorption selectivity should be used.

Membrane selectivity or the degree of separation is commonly defined by the relationship

$$S=\frac{(Y_B / Y_{1-B})}{(X_B / X_{1-B})}$$

Where B is the component of the mixture presenting pervaporation (acetone, butanol); Y is the fraction by weight of parameters; and X represents the fraction by weight in the

solution or fermentation medium. It was found that in all cases butanol flow is proportional to the butanol concentration in the solution i.e. greater the concentration, the greater the extraction. A similar, but less noticeable effect was seen in the case of acetone. In the case of ethanol and acids, their concentration in the solution remained constant. Thus the flow of ethanol and acids through the membrane at fermentation concentrations can be considered nil. The table below shows the selectivity of membrane for butanol and acetone. A greater selectivity can be seen for butanol over acetone.

Table 4.1 :- Flux and Selectivity values for Butanol

X	Y	Flow	S
0.0175	0.45	11.05	46
0.016	0.46	6.63	52
0.0148	0.46	5.30	57
0.014	0.45	4.42	58

Table 4.2 : Flux and Selectivity values for Acetone

X	Y	Flow	S
0.0064	0.18	4.42	34
0.0059	0.15	3.67	30
0.0055	0.15	2.65	32
0.0052	0.17	0.88	39

Another parameter that comes into picture during pervaporation through membrane is the flux which is given by the relation

$$\text{flux}=\frac{w}{At}$$

where w – weight of condensate in grams , A – membrane area , t – time for collection

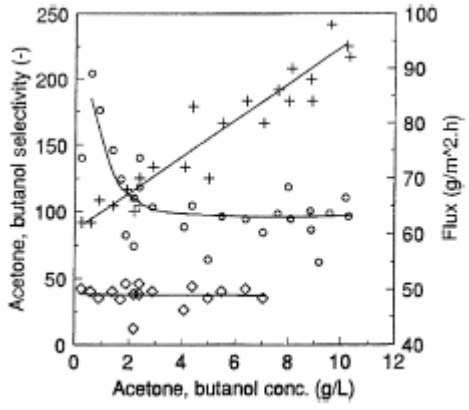

Fig 4.1:Graph showing the variation of flux and selectivity of Acetone, Butanol

The above graph shows that the separation factor S increases when fraction by weight of butanol decreases; the opposite effect takes place in the case of flow rate. This indicates that the membrane is more permeable to butanol.

In both the cases it was found that the biomass production reached a maximum value of 2.6g/L cells. The fermentation with pervaporation(FP) is completed in 40 h, while fermentation without pervaporation(FWP) requires 48 h. Glucose was completely consumed in both the cases. In FP, since no product inhibition was present, the microorganisms kept their metabolic activity ; i.e , the same glucose conversion was obtained in a shorter time than that required by FWP. With regard to products especially butanol, they showed similar evolution in both the cases, since butanol production rate was greater than the butanol extraction rate through pervaporation. However, when a butanol concentration of 6g/L was reached in the medium fermentation evolved differently in each case. This was due to the fact that at this concentration, the product inhibited the cells. Thus in FWP the rate of butanol production progressively decreased due to butanol's inhibiting effect on bacteria, until reaching a butanol concentration that halted growth completely(16g/L). However, in fermentation using pervaporation(FP), this effect was attenuated by *in situ* extraction of butanol through the membrane, thus keeping the butanol level lower than the inhibition concentration level at all times. The higher ethanol production in FP may be attributed to a better reutilization of acetate present in the fermentation medium to ethanol. It was also found that the pervaporation module did not affect the global yield of fermentation, but increased solvent production, mainly due to a total conversion of acids present in the solvents, since product-induced inhibition of cells did not take place.

Chapter 5

Model

Overview

5.1 Overview

A process oriented mathematical model of the acetone-butanol fermentation has to reflect the biochemical kinetics of the process and culture physiological aspects. Biosynthesis of solvents by *C. acetobutylicum* can be elucidated through many steps. The mechanism of glucose utilization by the culture, following the initial glycolytic steps, is proceeding toward the main end products formed on each side of the central cycle. Butanol as the main solvent end product is formed via a butyrate intermediate in the right branch of the pathway, while acetone is produced via acetate in the left branch, which is also concerned with the biosynthesis of ethanol. Gaseous by-products are generated in both branches as well as in the main line of the pathway before its branching. Knowledge of metabolic sequences is essential for formulation of mass balance equations whereby the "appearance" and "disappearance" of individual intermediates and products in the bioreactor system are quantitatively accounted.

Production strains of *C.acetobutylicum* are characterized by a relatively long lag phase, variable morphology during cultivation, pronounced sensitivity to pH, and a strong inhibition of growth and metabolic activities by butanol accompanied by cell lysis, or even sporulation. Based on the concept of the physiological applications, a conclusion has been made for an adequate mathematical description of the ABE process. A structured growth model has to be capable of at least partially describing the variability of the growth dynamics, which depends on the history of microbial culture. A suitable chosen marker of the physiological state can serve to introduce this unconventional aspect into the process model. A morphological image of the microbial population or a concentration of some intracellular component that significantly changes during the growth is used as a marker. Powell, in his theoretical work dealing with the modeling of transient states in the microbial culture, introduced an interesting possibility of modeling the relationship between the specific growth rate, the culture history and a variable environment using the *metabolic activity functional Q*. This relationship is defined as

$$\mu = Y_{X/S}\, Q\, g(S)$$

Where $Y_{X/S}$ is the theoretical, thermodynamically maximum macroscopic yield coefficient and g(S) is a simple function depending upon the environment. In a very simplified case this function is equal to unity when there is some substrate in the culture medium, while it becomes zero when there is none. It can be used for the simulation of transient states of the culture, which result from a perturbation imposing a stress on the microbial population. In the

case of fermentation considered here, it may be, the loss of anaerobic conditions, a substrate shock or another kind of perturbation resulting in destabilization of the culture physical equilibrium. In this context, the function is considered to be unity.

The metabolic activity functional Q is not a simple function of time, but its value depends on the culture history and variable substrate consumption rate during different stages of development of the microbial population. This functional has been defined by Powell as

$$Q(t) = \int_0^{g(S)} f(\varepsilon) q[S(t-\varepsilon)] d\varepsilon$$

Even though the metabolic activity functional has been formally derived from the variable morphological culture image, which depends on the culture age, according to Powell, it can be considered identical with a variation of a concentration of some cell component related to growth rate. The intracellular RNA concentration can be readily used for this purpose because RNA concentration exhibits a linear relationship with the cell growth rate, and because the ration of the individual RNA components is usually constant over a broad range of culture conditions. The relationship between growth rate and RNA can be expressed as

$$\mu = \text{const}(RNA - RNA_{min})\ g(S)$$

Where RNA_{min} is the RNA concentration in the cell at $\mu = 0$.

Considering the ABE process, it was established that the culture growth rate is directly proportional to the substrate consumption multiplied by a term characterizing the inhibition of culture growth by butanol:

$$\frac{d(RNAX)}{dt} = k_1 S \frac{K_I}{K_I + B}(RNAX)$$

The dimensionless concentration of RNA , designated as y, was used in this work as a marker of the culture physiological state, whereby

$$y = \frac{RNA}{RNA_{min}}$$

This way the specific growth rate of the culture, which has been shown to be related to the cellular RNA content, can be expressed as

$$\mu = ay - b$$

Harder and Roels demonstrated that the numerical values of the above coefficients are constant for most of the bacterial cultures. Consequently a parameter λ can be defined as

$$\lambda = const\frac{g(S)}{RNA_{\min}} = 0.56$$

which characterizes numerous bacterial cultures. Evaluation of culture dynamics associated with the marker of the culture physiological state can be performed also for C. acetobutylicum by expressing the following differential dimensionless balance:

$$\frac{d(yX)}{dt} = \mu(S,B)Xy$$

where the function μ(S,B) is a characteristic of the culture dependent on respective concentrations of the limiting substrate S and the inhibitory product B. The above equation can be mathematically rearranged to the following form:

$$\frac{dy}{dt} = \mu(S,B)y - 0.56(y-1)y$$

The initial condition for y(0) is y(0)=1, which characterizes the inoculum in its stationary phase. This initial condition characterizes the physiological state of the culture with regard to the previous culture history during the inoculum propagation. For the ABE process, a linear relationship with respect to substrate is combined with the simultaneous product inhibition of the Yerusalimski-Monod type. In expressing the differential equation for the biomass, the cell decay and lysis has to be considered which is directly proportional to the concentration of butanol(B) in the broth, the final equations assuming the following forms

$$\frac{dX}{dt} = \lambda(y-1)X - k_2XB$$
$$\frac{dy}{dt} = (k_1S\frac{K_I}{K_I + B} - \lambda(y-1))y$$

In writing the differential mass balance for the substrate, the sugar consumption for the production of acids and solvents are to be taken into consideration.

$$\frac{dS}{dt} = -k_3 SX - k_4 \frac{S}{K_S + S} X$$

A butyrate mass balance for the reaction system can be expressed as

$$\frac{dBA}{dt} = -k_5 S \frac{K_I}{K_I + B} X - k_6 \frac{BA}{BA + K_{BA}} X$$

In the mass balance the first term on the right hand side represents biosynthesis of butyrate from sugar substrate inhibited by butanol. The second term reflects the consumption of butyrate for its bioconversion into butanol. The terms K_I and K_{BA} are inhibition and saturation constants for the two reactions respectively. The differential mass balance for butanol in the system which enables simulation of the initial delay in the butanol production and accumulation caused by the intermediate accumulation of butyrate in the broth can be expressed as

$$\frac{dB}{dt} = k_7 SX - 0.841 \frac{dBA}{dt}$$

Coefficient 0.841 resulted from the stoichiometric conversion considerations as a ratio of molecular weights of butanol and butyric acid. The mass balance for acetic acid can be written as:

$$\frac{dAA}{dt} = k_8 \frac{S}{S + K_S} \frac{K_I}{K_I + B} X - k_9 \frac{AA}{AA + K_{AA}} \frac{S}{S + K_S} X$$

The dynamics of acetone, ethanol, carbon dioxide and hydrogen production can be expressed by the following equations:

$$\frac{dA}{dt} = k_{10}\frac{S}{S+K_S}X - 0.484\frac{dAA}{dt}$$

$$\frac{dE}{dt} = k_{11}\frac{S}{S+K_S}X$$

$$\frac{dCO_2}{dt} = k_{12}\frac{S}{S+K_S}X$$

$$\frac{dH_2}{dt} = k_{13}\frac{S}{S+K_S}X + k_{14}SX$$

Chapter 6

Solution to model

Without Pervaporation

With Pervaporation

6.1 Without Pervaporation

The differential equations obtained by taking into consideration the mass balance and the inhibitory effect by butanol were solved using the ode45 operator in Matlab. The initial conditions were suitably chosen so as to obtain our objective. Trial runs were also performed to see that the model worked for the conditions in the paper.

The solution code to the differential equations is given below

```
function  xdot = model( t,x ) ;
k1 = .009;
k2 = .0008;
k3 = .0255;
k4 = .6764;
k5 = .0135;
k6 = .1170;
k7 = .0113;
k8 = .7150;
k9 = .1350;
k10 = .1558;
k11 = .0258;
k12 = .6139;
k13 = .0185;
k14 = .00013;
ki = .11;
ks = 2.0 ;
kba =0.5 ;
kaa = 0.5 ;
d = 0.56;

y = x (1);
X = x (2);
s = x (3);
ba = x(4) ;
b = x (5);
```

```
aa = x(6);
a = x (7);
e = x (8);
c = x (9);
h = x (10);

dydt  = (((k1 * s *  ki )/( ki + b)) - ( d * ( y -1 ))) * y ;
dXdt = (( d  * ( y -1 ) * X )  - ( k2  * X * b ));
dsdt = ((-k3 * s * X ) -  ((k4 * s * X ) / ( ks + s )))  ;
dbadt = ((( k5  * s * ki * X )/ ( ki + b)) -(( k6 * ba * X )/ (kba + ba )) ) ;
dbdt = (( k7 * s * X ) - ( .841 * dbadt ));
aa1 = (( k8 * s * ki * X ) / (( ki + s ) * ( ks + b )) ) ;
aa2 = ((k9* aa * s * X )/ ((kaa + aa ) * (ks + s) )) ;
daadt = aa1 - aa2 ;
dadt = (((k10 * s * X)/(ks + s ))- ( .484 * daadt )) ;
dedt = ((k11 * s * X )/ ( ks + s ) ) ;
dcdt = (( k12 * s  * X )/  ( ks + s ) ) ;
dhdt = ( (( k13 * s * X ) / ( ks + s )) + ( k14 * s * X )) ;
xdot = [ dydt ; dXdt ; dsdt ; dbadt ; dbdt ;daadt ;dadt ; dedt ; dcdt ; dhdt  ] ;
```

In the matlab command window the code like the one given below may be suitably given so as to get the required output, i.e., the adequate number of plots in single run as well as displaying the values.

```
tspan = [ 0 80 ] ;
x0 = [ 1 ; .15 ; 100  ; 0 ;0;0;0;0;0 ;0 ];
[t,x] = ode45('model',tspan ,x0);
subplot(2,2,1),plot(t,x(:,5))
axis([0 40 0 30]);
subplot(2,2,2),plot(t,x(:,2))
axis([0 40 0 5]);
subplot(2,2,3),plot(t,x(:,3))
axis([0 80 0 100]);
```

The above commands in the command window plots the concentrations of butanol,biomass and substrate with time.

6.2 With Pervaporation

The solution code to this case may be obtained by the solution of the forementioned differential equations with the addition of a pervaporation factor for butanol and acetone. These factors were found out from the graph and were solved in the equations. The solution code to this condition may be given as

```
function  xdot = modifiedvalues1( t,x ) ;
k1 = .009;
k2 = .0008 ;
k3 = .0255 ;
k4 = .6764 ;
k5 = .0135 ;
k6 = .1170 ;
k7 = .0113 ;
k8 = .7150 ;
k9 = .1350 ;
k10 = .1558 ;
k11 = .0258 ;
k12 = .6139 ;
k13 = .0185 ;
k14 = .00013 ;
ks = 2.0 ;
kba =0.5 ;
kaa = 0.5 ;
d = 0.56 ;
y = x(1) ;
X = x(2) ;
s = x (3) ;
ba = x(4) ;
b = x(5);
aa = x(6);
```

```
a = x(7);
e = x(8) ;
c = x(9);
h= x(10);
v = 1.5 ;

rp =  0.004 *  b ^ 3.5411 * .0628 * .75    ;

ki = .08;

dydt  = ((((k1 * s * ki )/(ki + b)) - (d  * ( y -1 ))) * y   );
dXdt = (( d * ( y -1 ) * X )  - ( k2 * X * b ))  ;
dsdt =  ((-k3 * s * X ) -  ((k4 * s * X ) / ( ks + s ))) ;
dbadt = ( ((k5 * s * ki * X )/(ki + b )) + (( -k6 * ba * X )/ (kba + ba )) );
dbdt = ((( k7 * s * X ) - ( .841 * dbadt ))  - ( (  rp    ) / v )) ;
aa1 = (( k8 * s * ki * X ) / (( ks + s ) * ( ki + b )) ) ;
aa2 = (( k9 * aa * s * X )/ ((kaa + aa ) * (ks + s) )) ;
daadt =  aa1 - aa2  ;
dadt = (((k10 * s * X )/( ks + s ))- ( .484 * daadt ) - ( ( rp1 ) / v )) ;
dedt =  ((k11 * s * X )/ ( ks + s ) )  ;

dcdt = (( k12 * s  * X )/  ( ks + s ) ) ;
dhdt = ( (( k13 * s * X ) / ( ks + s )) + ( k14 * s * X )) ;
 xdot = [  dydt ; dXdt ; dsdt ;dbadt ; dbdt ;daadt ; dadt ; dedt ;dcdt ;dhdt       ] ;
```

The corresponding code in the command window may be given as

```
% values for the acetone and butanol
tspan = [ 0 80 ];
x0 =[  1 ; .15  ; 100 ;0 ;0 ; 0  ];
[t,x] = ode45('modifiedvalues1',tspan,x0);

subplot(3,3,2),plot(t,x(:,5))
subplot(3,3,3),plot(t,x(:,3))
subplot(3,3,4),plot(t,x(:,2))
```

Chapter 7

Result and Discussion

7.1 Result and discussion

The above code was run for the different values of substrate , biomass and RNA concentrations and the results obtained were analyzed. The general nature of the curves with and without pervaporation is shown below .

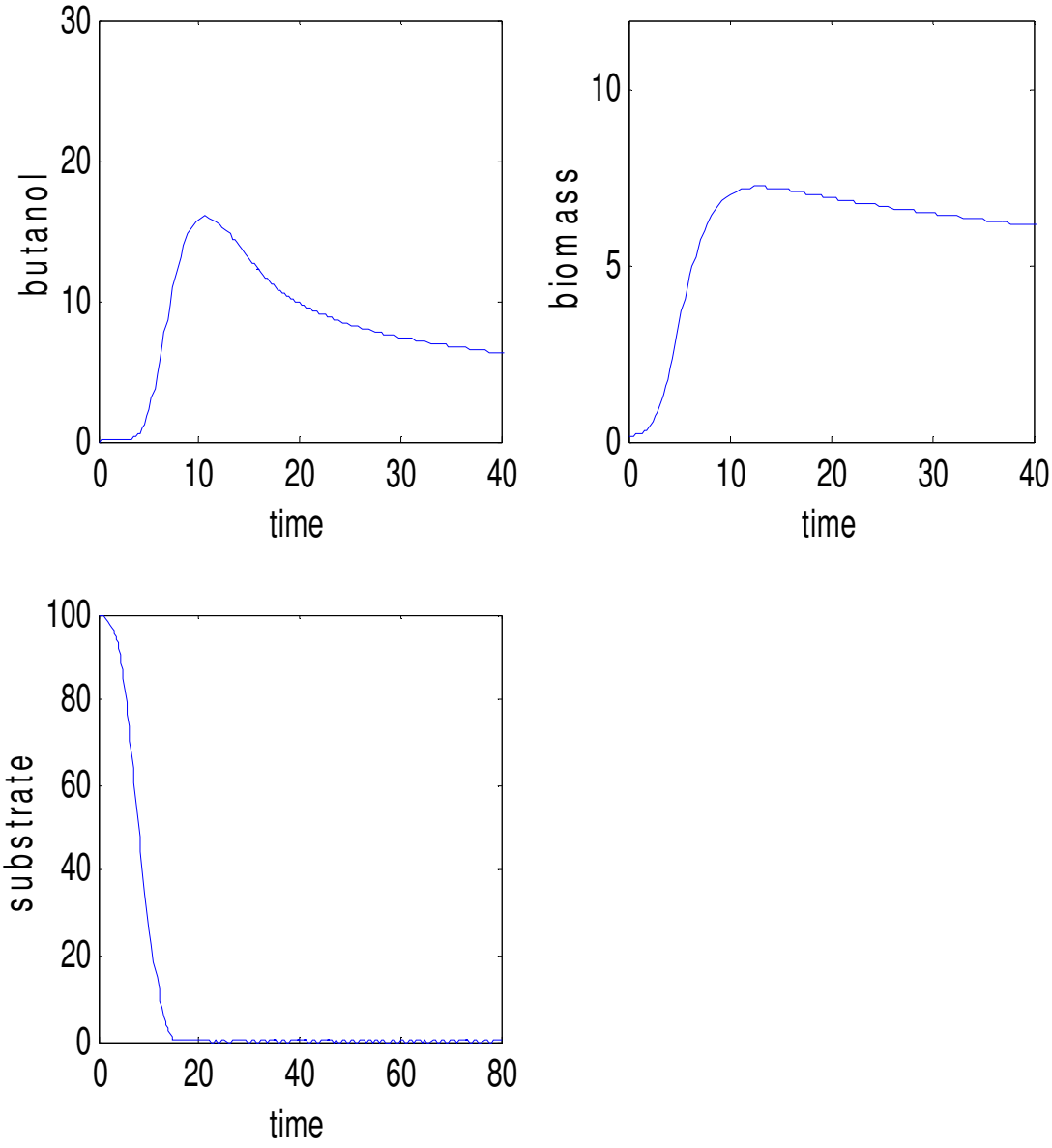

Fig7.1 : curves for process with pervaporation

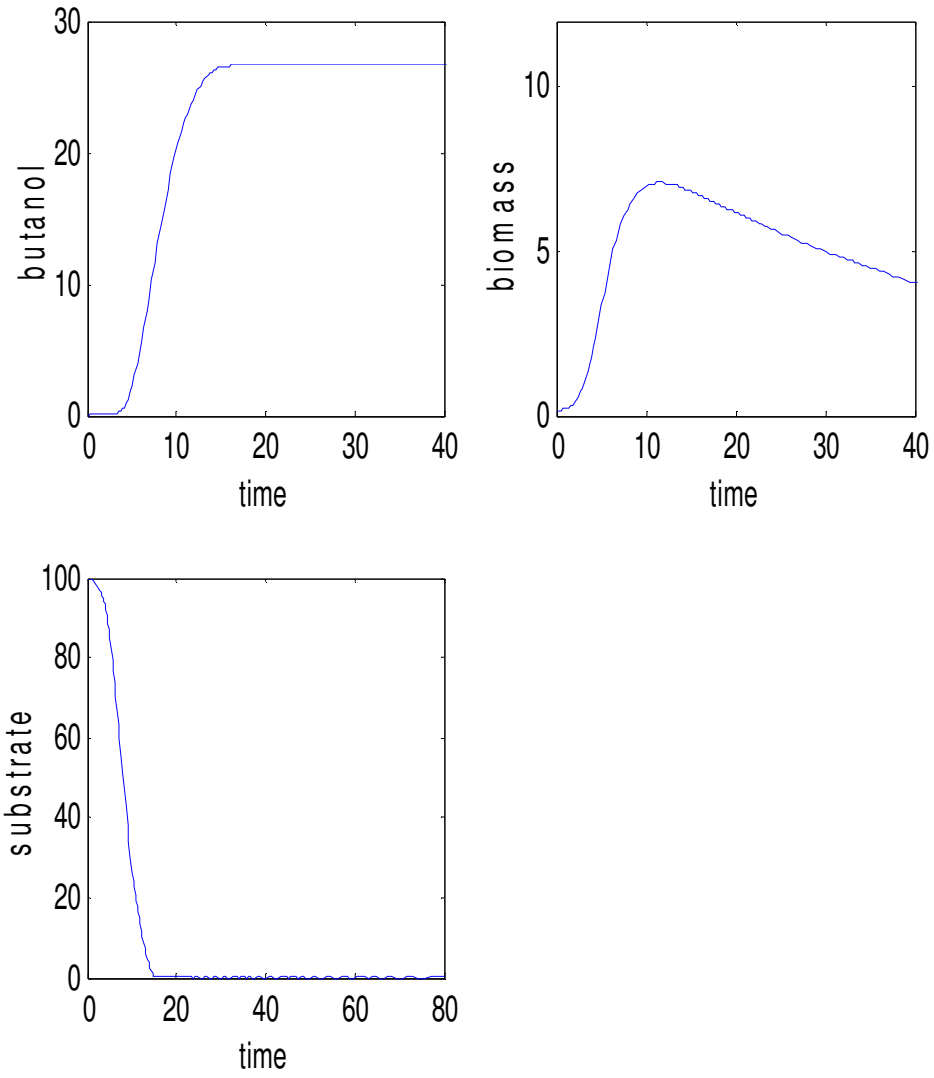

Fig7.2:curves for process without pervaporation

But here we see that the substrate is getting consumed around the time span in both the process. So we play with the inhibition constant so as to get the required condition.

7.1.1 Changes of Substrate consumption with Inhibition constant (K_I)

Here we attempt to find the change of substrate consumption so as to get a general idea about the effect of inhibition constant on the process.

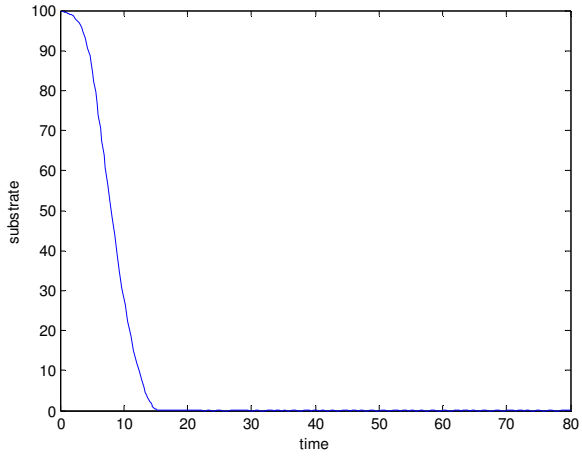

Fig:7.3(a)

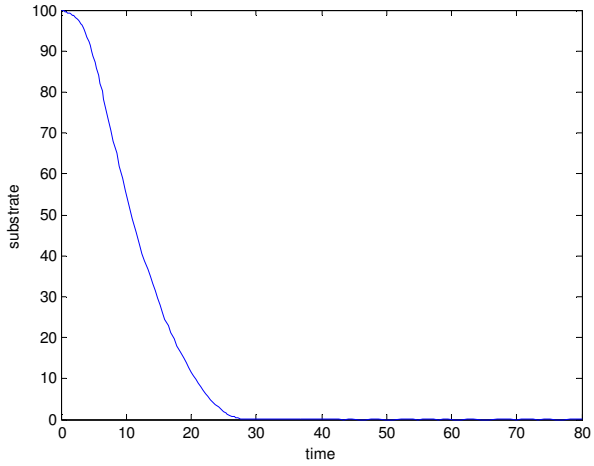

Fig: 7.3(b)

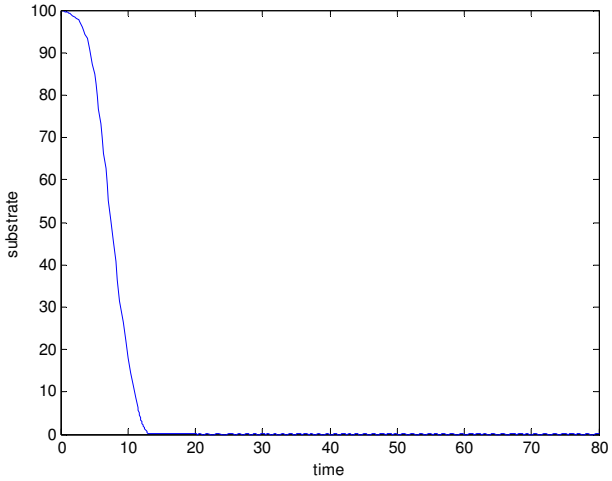

Fig7.3(c)

Fig 7.3(a) with K_I = .833 (b) with K_I = .3 (c) with K_I = 1.2

From the above curves we find that the substrate consumption rate is inversely proportional to the values of K_I

7.1.2 Changes of Biomass Production with inhibition constant

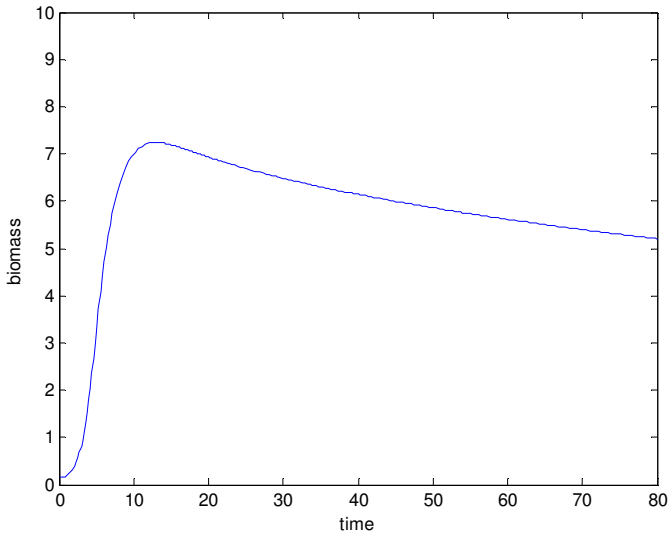

Fig 7.4(a)

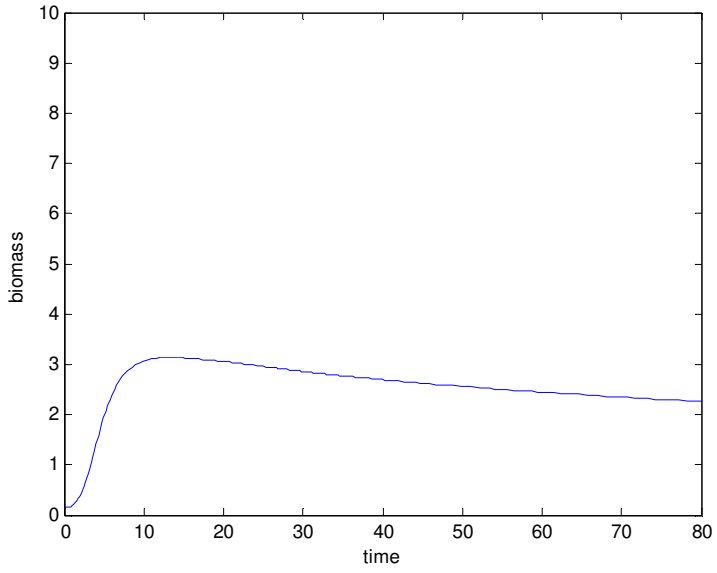

Fig 7.4(b)

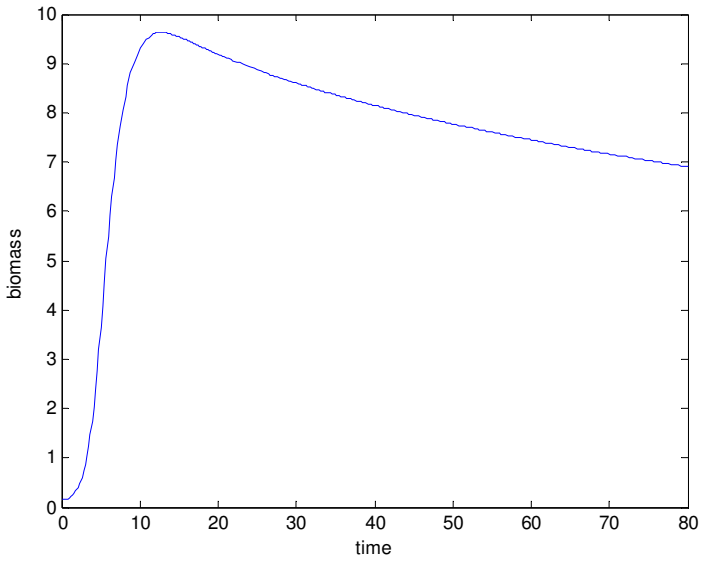

Fig 7.4 (C)

Fig 7.4(a) with K_I = .833 (b) with K_I = .3 (c) with K_I = 1.2

So from the above curves it is really evident that the biomass production is directly linked to the value of the inhibition constant, more the value of ki, more the maximum amount of biomass produced.

7.1.3 Changes in Butanol Production with changes in K_I

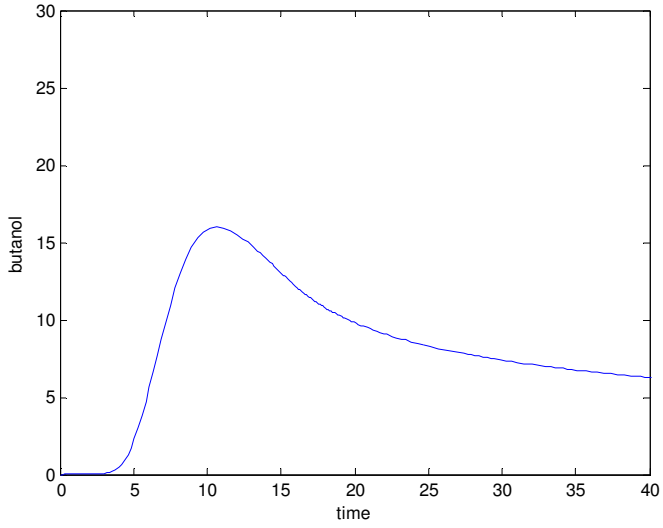

Fig 7.5(a)

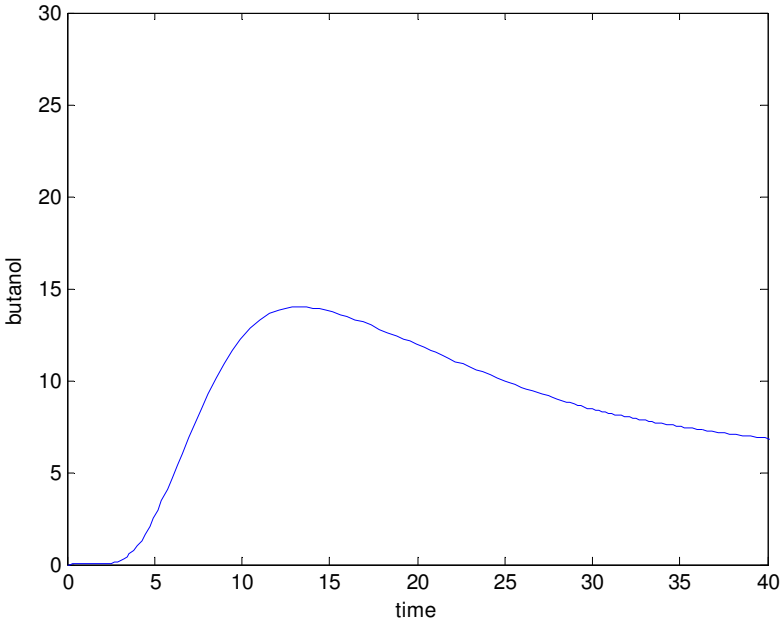

Fig 7.5(b)

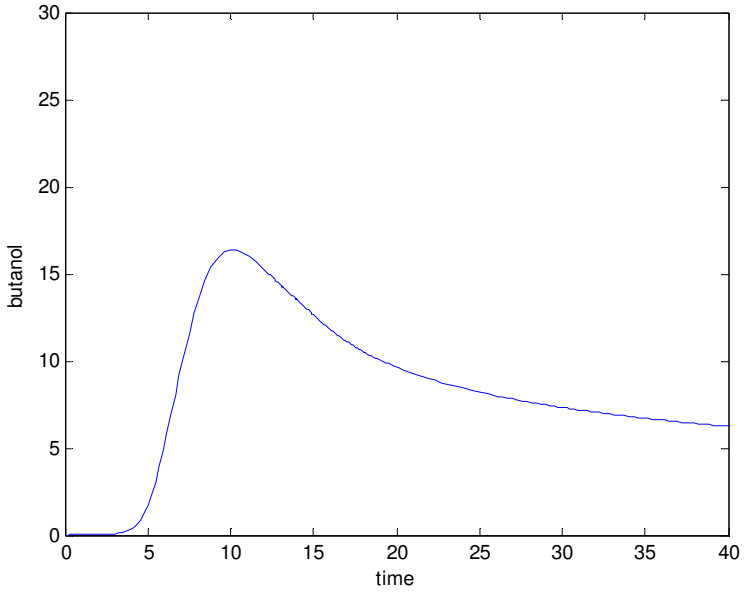

Fig 7.5(c)

Fig7.5(a) with K_I = 0.833 (b) with K_I = 0 .3 (c) with K_I = 1.2

It can be found that the butanol production is relatively unaffected by the changes in the value of the inhibition constant and it tends to a relative minimum at the optimum value of K_I = 0.833

7.1.4 Effect of inhibition constant on the two models

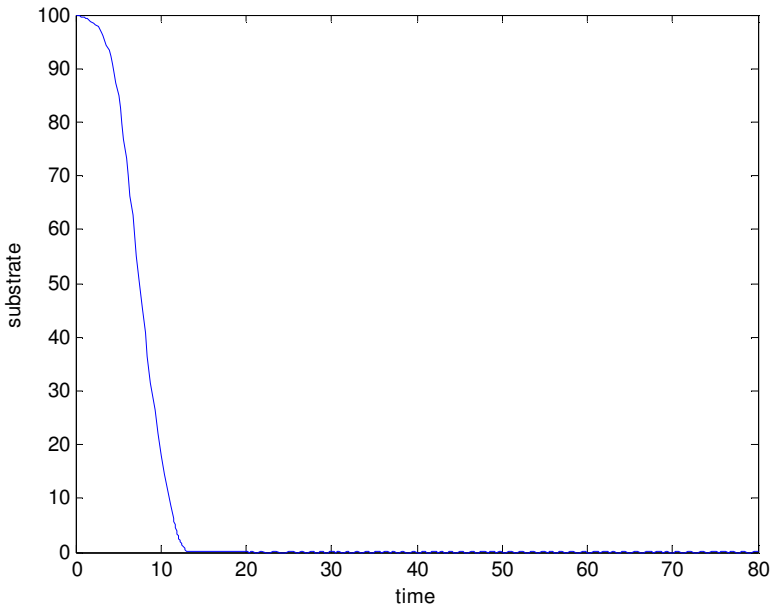

Fig7.6(a)

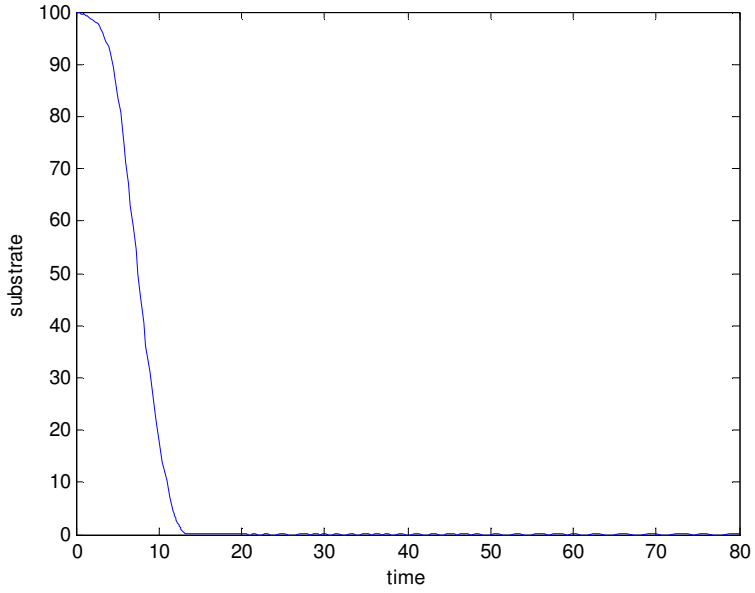

Fig 7.6(b)

Fig7.6 (a)model with pervaporation (b) model without pervaporation

It can be seen that in both the cases the time required for the substrate to reduce to zero value is around the same. It may also be noted that there is no unconverted substrate in the case of model without pervaporation although it is not the case though. So the model fails in this aspect. At this juncture we fix up the problem by using different values of K_I in the two papers or the value of K_I needs to be optimized. So we played with the constant K_I to get an

approximate representation of the plots in the process and the model paper. After several runs the value of K_I could be fixed as K_I = 0.029 so that we could get an approximate representation.

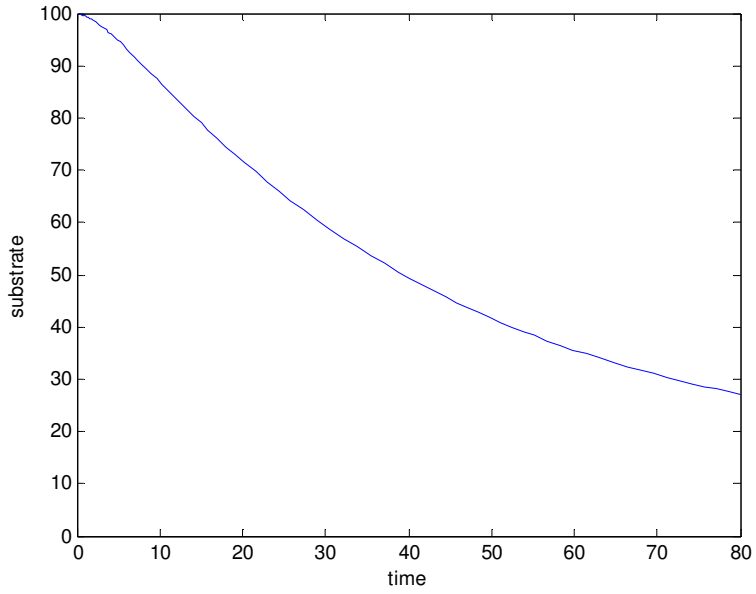

Fig 7.7 (a) model without pervaporation

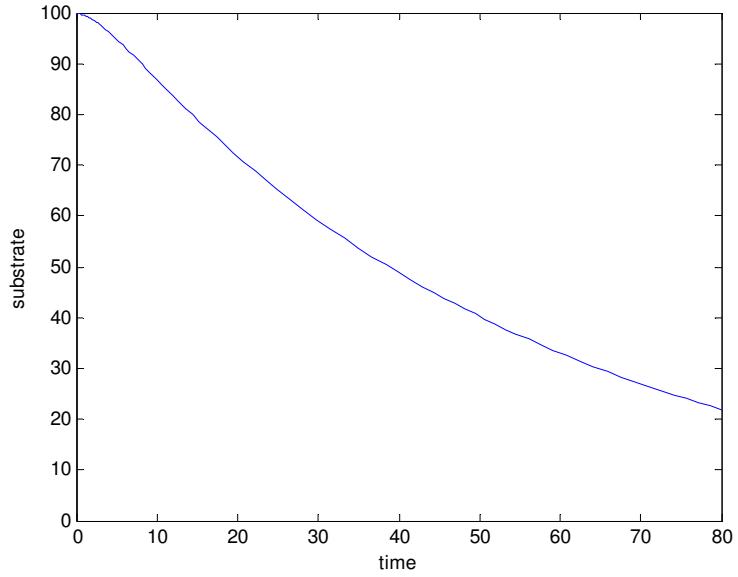

Fig7.7(b) model with pervaporation

Fig 7.7:Plots of the models after fixing inhibition constant

7.1.5 General Representation of the Products

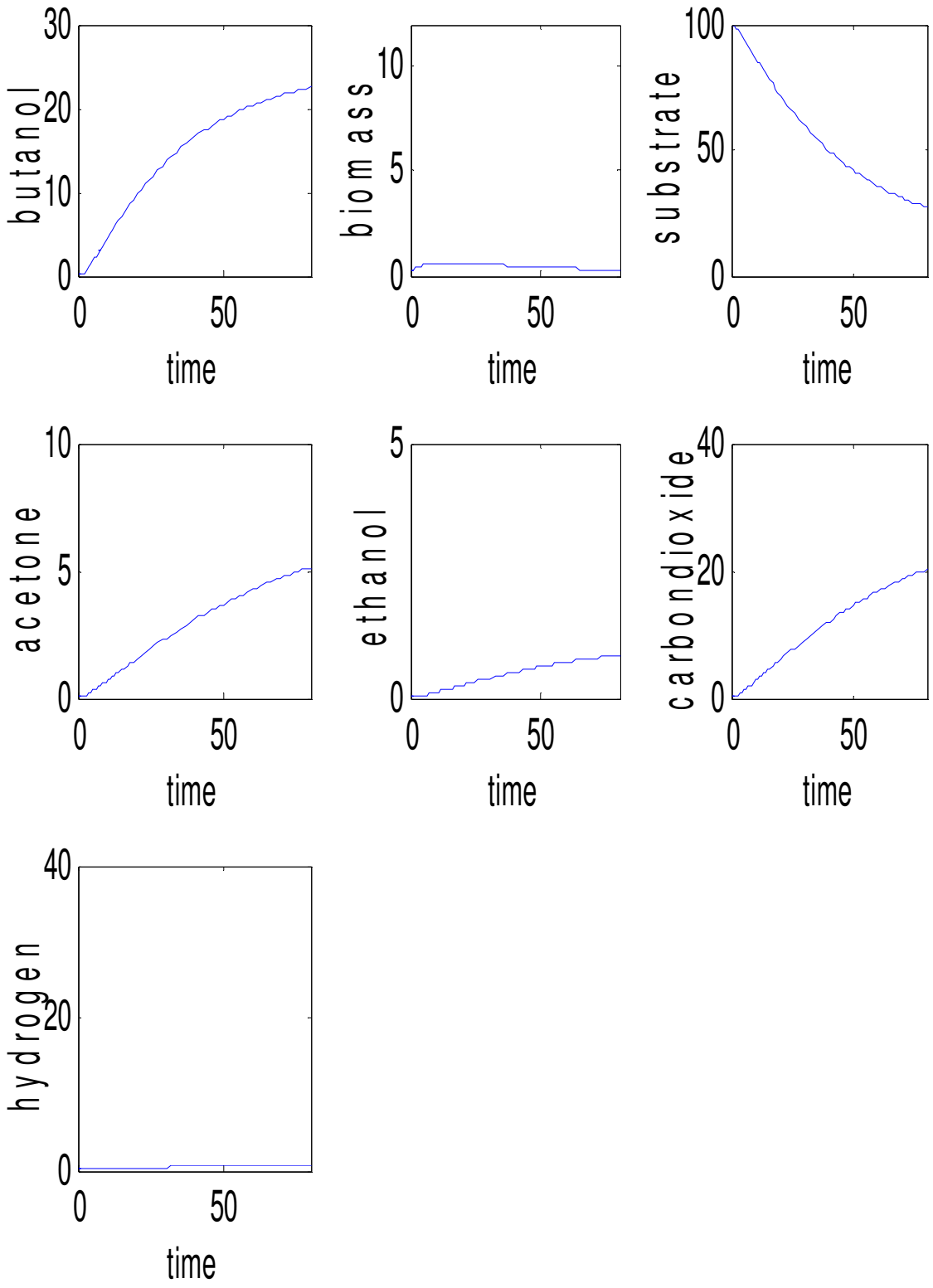

Fig7.8(a) model without pervaporation

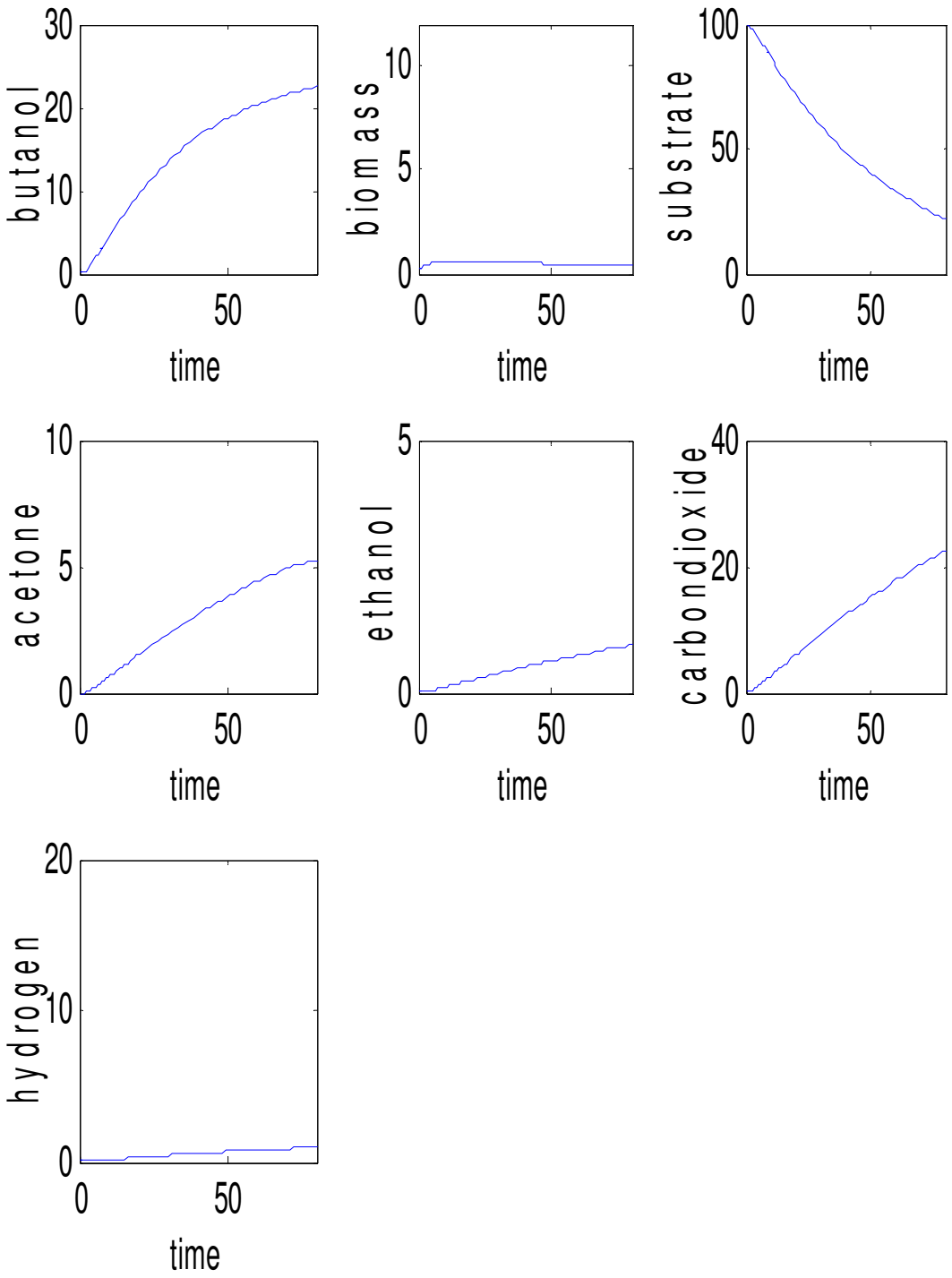

Fig7.8(b) model with pervaporation

So the only method is to use different values of K_I in both the models so as to get an approximate reproduction of the plots in both the model and the process paper.

Chapter 8

Conclusion

8.1 Conclusion

The summary of the project can be concluded in the following points :

- the value of the inhibition constant had a pronounced effect on the amount of biomass production and the substrate consumption rate
- changing the value of the inhibition constant drastically result in changes affecting the amount of butanol produced.
- There was no change in the production of ethanol, acetone , carbondioxide and hydrogen in both the cases although some changes in the production of solvents were expected
- The biomass production reached the same maximum value in both the cases (but less value than the paper), a fact supporting the model
- The rate of pervaporation obtained was low and it may have significant effect on the amount of butanol produced
- Although the relationship for the rate of pervaporation was obtained from the paper by using the given data , but inherent factors like membrane scaling were not considerer which would often contribute to errors. The rp factor has to be corrected further for these inherent factors

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